

## CLAIMS

The invention claimed is:

1. A method for determining the extent of differentiation in a population of isolated human embryonic stem (hES) cells, comprising detecting or measuring two or more markers preferentially expressed in undifferentiated hES cells, and one or more markers preferentially expressed after differentiation of the hES cells.
2. The method of claim 1, wherein at least one of the markers preferentially expressed in undifferentiated hES cells is selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), and human telomerase reverse transcriptase (hTERT).
3. The method of claim 1, wherein at least one of the markers preferentially expressed in undifferentiated hES cells is selected from Oct 3/4, SSEA-4, and the markers detected by antibodies Tra-1-60 and Tra-1-81.
4. The method of claim 1, comprising measuring three or more markers preferentially expressed in undifferentiated hES cells selected from hTERT, Oct 3/4, Cripto, GRP, PODXL, SSEA-3, SSEA-4, Tra-1-60 and Tra-1-81.
5. The method of claim 1, comprising detecting or measuring hTERT, Oct 3/4, and a marker selected from Cripto, SSEA-4, Tra-1-60 and Tra-1-81.
6. The method of claim 1, wherein at least one of the markers preferentially expressed after differentiation of the hES cells is a stromal cell markers.
7. The method of claim 1, wherein the stromal cell marker is selected from CD44, CD105 (endoglin), CD106 (VCAM-1), CD90 (Thy-1), STRO-1, Vimentin, and Human Thymus Stroma.
8. The method of claim 1, wherein expression of hTERT, Oct 3/4, Cripto, GRP receptor, PODXL, CD44, CD105, CD106, or CD90 is detected or measured at the mRNA level by PCR amplification.
9. The method of claim 8, wherein the measuring at the mRNA level is conducted by real-time PCR amplification.
10. The method of claim 1, wherein expression of SSEA-3, SSEA-4, Tra-1-60, Tra-1-81, Cripto, Oct 3/4, CD44, CD105, CD106, CD90, STRO-1, Vimentin, or Human Thymus Stroma is detected or measured at the antigen expression level by antibody assay.

11. The method of claim 10, wherein the measuring at the antigen expression level is conducted by flow cytometry using fluorescence-labeled antibody.
12. The method of claim 10, wherein the measuring at the antigen expression level is conducted by immunocytochemistry.
13. The method of claim 1, comprising measuring some of said markers at the mRNA level, and some of said markers at the antigen expression level.
14. The method of claim 1, comprising quantifying the proportion of undifferentiated hES cells or differentiated cells in the culture from said marker expression according to positive expression of the undifferentiated cell markers, and lack of expression of the stromal cell markers.
15. The method of claim 1, comprising assessing the ability of a soluble factor or culture medium to maintain hES cells in an undifferentiated state from said marker expression.
16. The method of claim 1, comprising assessing the suitability of an undifferentiated hES cell population for preparing differentiated cells for human administration.
17. A system for assessing a culture of undifferentiated human embryonic stem (hES) cells or their progeny according to claim 1, comprising antibody or PCR amplification primers specific for three or more markers, of which at least two are preferentially expressed in undifferentiated hES cells, and at least one is preferentially expressed in stromal cells.
18. The system of claim 17, comprising antibody or PCR amplification primers specific for at least two markers selected from Cripto, gastrin-releasing peptide (GRP) receptor, podocalyxin-like protein (PODXL), human telomerase reverse transcriptase (hTERT) Oct 3/4, SSEA-4, and the markers detected by antibodies Tra-1-60 and Tra-1-81.
19. The system of claim 17, comprising antibody or PCR amplification primers specific for at least one stromal cell marker selected from CD44, CD105 (endoglin), CD106 (VCAM-1), CD90 (Thy-1), STRO-1, Vimentin, and Human Thymus Stroma.
20. The system of claim 17, packaged as a kit with instructions for using the components of the kit for assessing a culture of undifferentiated human embryonic stem (hES) cells.